Growth and Telomerase Inhibition of SK-MEL 28 Melanoma Cell Line by a Plant Flavonoid, Apigenin

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The plant flavonoids, including apigenin which is found in especially high concentrations in edible plants, are reported to protect against chronic diseases including several types of cancer. We observed that apigenin inhibited not only the growth of human melanoma cell line SK-MEL 28 but also the telomerase activity. We also noted the telomerase activity inhibition by genistein, a tyrosine kinase inhibitor found in soybean, which suggests that the telomerase activity of SK-MEL 28 cells may be regulated by the protein phosphorylation. Furthermore, we observed that apigenin induced SK-MEL 28 cell apoptosis with p53 up-regulation. Taken together, our results indicate that apigenin plays a role as an antimelanoma component in edible plants.

Introduction

Apigenin, one of the flavonoids which are widely distributed in edible plants, has been shown to inhibit the growth of a number of cancer cells *in vitro* and *in vivo* (Plaumann *et al.*, 1996; Fotsis *et al.*, 1997). Apigenin has also shown a strong sunscreen effect by ultraviolet light absorption and an anticancer effect by the interference with the tyrosine kinase which is one of the important enzymes for cell growth (Klein *et al.*, 1996; Birt *et al.*, 1997). Apigenin blocks several points in the process of tumor promotion with the inhibition of protein kinases and the reduction of transcription factors, resulting in the regulation of the cell cycle. Apigenin induces G1 cell-cycle arrest with the activation of the cdk inhibitor p21/WAF1, resulting in the cdk2 kinase activity inhibition and the

accumulation of the hypophosphorylated form of the retinoblastoma protein in a dose-dependent manner (Kuo and Yang, 1996; Lepley *et al.*, 1996; Lepley and Pelling, 1997). Apigenin is also reported to induce apoptosis, a programmed cell death which is a physiological mechanism for the regulation of tissue homeostasis (Plaumann *et al.*, 1996; Levine, 1997). The regulation of apoptosis is a complex process including p53 and a number of cellular genes (Levine, 1997). p53 is known as a gate-keeping protein to protect DNA damage from the hazardous stimuli with its various functions, including the promotion of apoptosis and the transcriptional activation of several genes (Lee *et al.*, 1997). However, it still remains to be characterized how apigenin promotes the cancer cell apoptosis.

Telomerase, a RNA/protein complex, catalyzes the telomere (the ends of mammalian chromosomal DNA) elongation. It has been proposed that the length of telomere regulates cell proliferation (Rhyu, 1995; Bestilny *et al.*, 1996). In somatic cell divisions, telomeres become shorten until the cells reach a critical point where some cellular factor(s) recognize(s) the shortened telomeres. After passing the M1 crisis, most cells enter the senescence stage. The cell in which genetic mutations are accumulated grows a clonal population to continue to divide and escapes senescence, resulting in a further telomere loss. After further cell divisions, most cells reach M2 crisis and die. A rare cell among them which acquires its telomerase activity somehow results in the stabilization of its telomere length and grows as a cancer. Thus, the telomerase activation during immortalization seems to be related with the development of cancer (Holt *et al.*, 1996). The abnormally active telomerase in cancer cells has become one of the most interesting targets to cure many cancers. It is, however, at the moment less studied on how telomerase is regulated in human cancers. To our knowledge, there was no report yet on whether apigenin affects the human telomerase activity.

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Considering these evidences together, we have recently been interested in the growth inhibition of the human melanoma cell line by apigenin as an anticancer agent. In the preliminary study, we found that the SK-MEL 28 cell line had an abnormal telomerase activity. Thus, we have asked the question of how apigenin affects the expression of p53 and the telomerase activity in SK-MEL 28 cells. In this study, we have observed that apigenin inhibits SK-MEL 28 cell growth, inducing apoptosis and morphological changes in a dose-dependent manner. Our results also demonstrate, for the first time, that apigenin inhibits the telomerase activity in a time- and dose-dependent manner.

Materials and Methods

Cell culture SK-MEL 28 cell (a human melanoma cell line) was purchased from ATCC, Rockville, USA (Brown et al., 1981). Media and supplements were obtained from GIBCO, Grand Island, USA. Cells were maintained in Dulbecco's Modified Essential Medium (DMEM) containing 10% heat-inactivated (30 min at 56°C) fetal bovine serum (FBS), 100 U/ml potassium penicillin, 100 μg /ml streptomycin, 2 mM glutamin, and 20 mM sodiumbicarbonate. The cells were incubated at 5% CO_2, 95% humidity, and 37°C. The growth media was changed every 3 days.

Cell growth study SK-MEL 28 cells (1×10^4) were placed into 24-well plates and grown in 10% FBS DMEM for 3 days. When the cell's confluence became 50%, media were changed with 10% FBS DMEM containing apigenin (obtained from Calbiochem-Novabiochem. Co., La Jolla, USA) at 0, 45, 100, and 180 μM, or DMEM without serum for the control experiment. The plates were incubated for 6, 24, 48, and 72 h in a final volume of 200 μl culture medium per well. The cell growth study was performed with a Cell Proliferation Kit 1 (MTT) purchased from Boehringer Mannheim (Mannheim, Germany).

Morphology change study SK-MEL 28 cells (1×10^4) were placed in 24-well plates and grown in 10% FBS DMEM for 3 days. When the cell was 50% confluent, the media were changed with 10% FBS DMEM containing apigenin at 0, 45, 100, and 180 μM, or DMEM without serum for the control. The plates were incubated for 24 h. The morphology of the cells was photographed with the inverted microscope (Nikon, FMS) equipped with a camera (Nikon, DHX-II) at 200X magnification.

Telomerase assay SK-MEL 28 cells (1×10^6) were plated into 100-mm^2 plates and grown in 10% FBS DMEM for 3 days. When the cell's confluence became 70%, media were changed with 10% FBS DMEM containing apigenin at 0, 45, 100, and 180 μM, or DMEM without serum for the control experiment. For the other sets of plates containing 70% confluent cells, media were changed with 10% FBS DMEM containing genistein (obtained from Calbiochem-Novabiochem. Co., La Jolla, USA) at 0, 45, 100, and 180 μM. After the plates were incubated for 6, 24, 48, and 72 h, the cells were harvested and the cell number was counted. The counted cells were centrifuged at $3000 \times g$ for

10 min at 4°C. For the each reaction, 2×10^6 cells were transferred into a fresh Eppendorf tube. The telomerase-reaction mixture with the cell extract (corresponding to 5×10^3 cells or 2 μg protein) was amplified by the telomeric repeat amplification protocol (Kim and Wu, 1997). The assay procedure for telomerase was followed using the protocol booklet provided by the Telomerase PCR ELISA manufacturer, Boehringer Mannheim (Mannheim, Germany). The optical density (OD) of the samples was measured with a microtiter plate reader at 450 nm within 30 min after addition of the stop solution.

Apoptotic DNA fragmentation The cell growth condition was the same as the telomerase assay described above, except for the apigenin treatment for 24 h. The harvested cells were centrifuged at $3000 \times g$ for 10 min at 4°C. The pelleted cells were resuspended in 200 μl of phosphate buffer. The apoptotic DNA ladder assay was performed with the procedure previously described (Baek et al., 1996; Kim et al., 1996). We used the Apoptotic DNA Ladder kit purchased from Boehringer Mannheim (Mannheim, Germany) for this experiment. For DNA gel electrophoresis, 2% agarose DNA gel (in 1X TAE with EtBr) was prepared. Approximately, 2 μg of purified DNA with 6X loading buffer was loaded using a micropipette. The gel electrophoresis was carried out for 30 min at 50 mV.

Western blot p53 antibody was purchased from Calbiochem (La Jolla, USA). The Western blot was performed with the procedure previously described (Kang and Folk, 1992). The primary antibody for p53 was diluted 1/5000 in the blocking buffer. The blot was incubated with diluted primary antibody for 1 h (10 ml). It was then washed twice for 5 min in the wash buffer (20 ml per wash). Western Star Chemiluminescent Detection System obtained from Tropix (Bedford, USA) was used for the detection.

Results

SK-MEL 28 cell growth inhibition and the induction of the cell morphology change by apigenin As an initial step to determine whether apigenin inhibits the melanoma cell growth in vitro, we used SK-MEL 28 cells grown in DMEM with 10% FBS containing 0, 45, 100, and 180 μM apigenin for 6, 24, 48, and 72 h, and counted the cell number with the MTT method as described in Materials and Methods. We observed that the cell growth was inhibited 100% at high apigenin concentration (100 μM) for 72 h. The cell number with the treatment of 45 μM apigenin for 72 h was 50% of that in the normal growth condition (Fig. 1). We, however, did not observe the cell growth inhibition by apigenin at less than 45 μM (data not shown). Thus, this result strongly suggests that apigenin (at concentrations higher than 45 μM) inhibits the human melanoma SK-MEL 28 cell growth. To compare the apigenin effect on SK-MEL 28 cell growth, we also examined the cell growth in the serum-free growth media. We observed that SK-MEL 28 cells grew in the serum-free condition with the cell doubling time of 48 h.
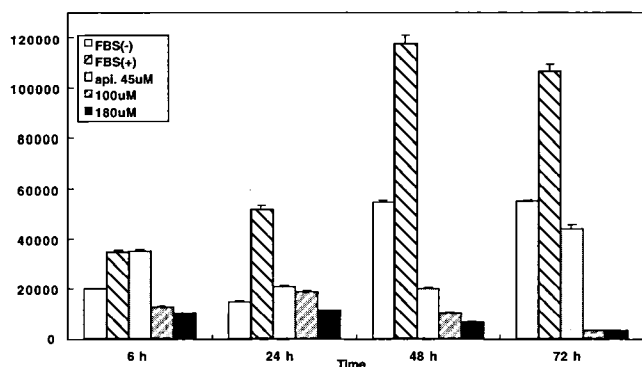
Fig. 1. SK-MEL 28 cell growth inhibition by apigenin. SK-MEL 28 cells (1×10^4) were plated into 48-well plates and grown in 10% FBS DMEM for 3 days. When the cells were 50% confluent, media were changed with 10% FBS DMEM containing 0, 45, 100, and 180 μM apigenin or DMEM without FBS for the control. The cell growth for 6, 24, 48, and 72 h was determined by the MTT method as described in Materials and Methods. The values are the mean of four replicates. Each bar indicates the standard error. We observed that the cell growth was inhibited by apigenin in a time- and dose-dependent manner and the cell doubling time in the serum-free media was 72 h.

To determine whether apigenin induces a change in cell morphology, we compared it in the growth media containing apigenin at 0, 45, and 100 μM for 24 h, or in DMEM without serum for the control experiment (Fig. 2). The cell morphology was dramatically changed in a dose-dependent manner. At the high concentration of apigenin (100 μM), the cell morphology was changed to the characteristic apoptotic cell shape.

Telomerase activity inhibition by apigenin In the preliminary experiment, we observed that the telomerase of SK-MEL 28 cells was highly activated. To determine whether apigenin inhibits the telomerase of SK-MEL 28 cell, we examined the telomerase of SK-MEL 28 cells grown in DMEM 10% FBS containing apigenin at 0, 45, 100, and 180 μM, for 24, 48, and 72 h, using the PCR ELISA method. Positive control was 293 cells provided by the manufacturer. Negative control was heat-treated (65°C, 10 min) SK-MEL 28 cells. As the control experiment, we also examined the telomerase of SK-MEL 28 cells grown in the serum-free media.

When the cells were grown in the media containing 45 μM apigenin for 72 h, SK-MEL 28 cell telomerase activity $(OD_{450} 2.3)$ was reduced at two thirds of the maximum telomerase activity $(OD_{450} 3.1)$ of the cells grown in normal growth condition (Fig. 3). Furthermore, the human melanoma telomerase was clearly inhibited by apigenin at the high concentration (180 μM) from OD_{450} 2.6 for 24 h to OD_{450} 1.2. for 72 h, in a time-dependent manner.
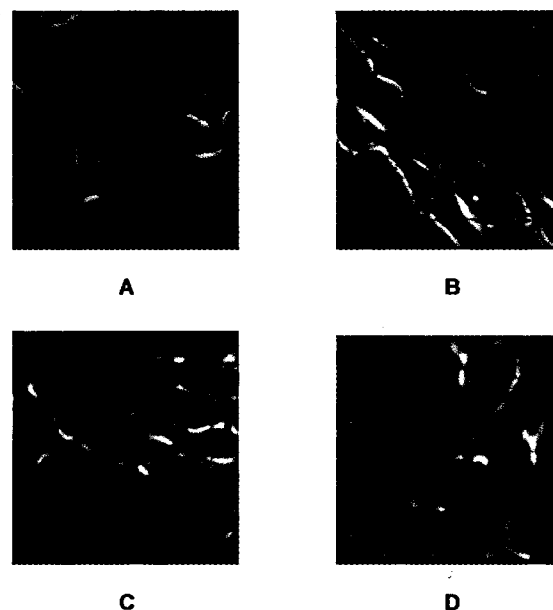

Fig. 2. SK-MEL 28 cell morphology change by apigenin. The cells were grown and treated apigenin in the same condition described in Fig. 1, except that the plate incubation was for 24 h. A, media without FBS for the control; B, media with 10% FBS without apigenin; C, media with 10% FBS containing 45 μM apigenin; D, media with 10% FBS containing 100 μM apigenin. The photograph for cells was taken by the inverted microscope (Nikon, FMS) equipped with camera (Nikon, DHX-II) at 200 times magnification. We observed that the cell morphology was changed to the apoptotic cell shape by the apigenin treatment (100 μM).

The telomerase activity of SK-MEL 28 cells grown in the serum-free media was significantly maintained at a minimum level, at OD_{450} 2.0 for 24 h, and that was increased to OD_{450} 3.5 and OD_{450} 3.6 for 48 h and 72 h, respectively (Figs. 3 and 4). Thus, the telomerase activity seemed to be reactivated to the maximum level in the serum-free media after 48 h. It was consistent with SK-MEL 28 cell doubling time (48 h) in serum free-media (Fig. 1). Interestingly, the telomerase activity $(OD_{450}$ 3.5 and OD_{450} 3.6 for 48 h and 72 h, respectively) in the serum-free media was higher than that $(OD_{450}$ 3.1 and OD_{450} 3.3 for 48 h and 72 h, respectively) grown in normal growth condition, even though the cell growth in the serum-free media was one thirds of that in the normal media (Fig. 1).

To examine whether a known tyrosine kinase inhibitor also inhibits the telomerase of SK-MEL 28 cell, we measured the telomerase activity of SK-MEL 28 cells grown in the presence of genistein. It has been reported that genistein, one of two major soybean isoflavonoids which are economically important, has a strong anti-angiogensis and anticancer effect by the interference with tyrosine kinase, which is one of the important enzymes for cancer cell growth (Kennedy, 1995; Brandi, 1997; Rauth, *et al.,*
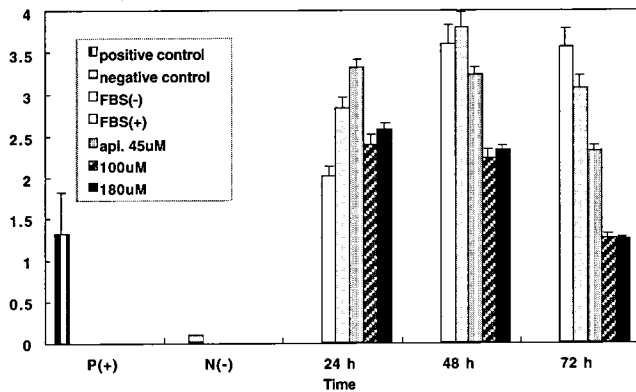
Fig. 3. The telomerase activity inhibition by apigenin. The cells (1×10^6) were placed into 100 mm^2-culture dish and grown in 10% FBS–DMEM for 3 days. When the cells became 70% confluent, media were changed with 10% FBS–DMEM containing 0, 45, 100, and 180 μM apigenin, or DMEM without FBS for the control experiment. The telomerase-reaction mixture with the 24, 48, and 72 h culture cell extract (corresponding to 5×10^3 cells) was amplified, following the telomeric repeat amplification protocol provided by Telomerase PCR ELISA manufacturer, Boehringer Mannheim (Mannheim, Germany). Positive control was 293 cells provided by the manufacturer. Negative control was heat-treated (65°C, 10 min) SK-MEL 28 cells. The values are the mean of three replicates. Each bar indicates the standard error. We observed that the telomerase activity was inhibited by apigenin in a time- and dose-dependent manner.
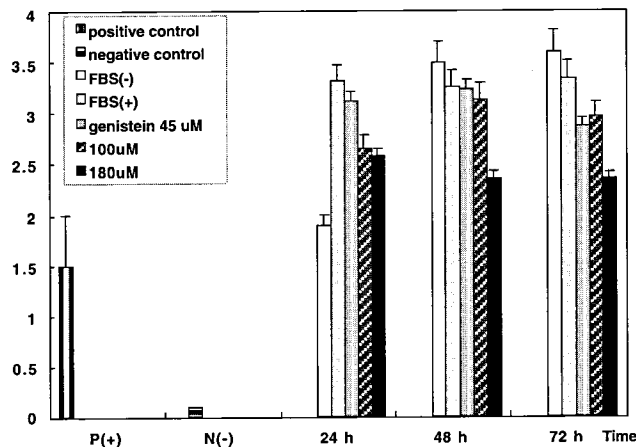
Fig. 4. The telomerase activity inhibition by genistein. The cells (1×10^6) were grown in the same conditions as described in Fig. 3, except that genistein was added in the media. The telomerase activity was measured with the Telomerase PCR ELISA method described in Fig. 3. The values are the mean of three replicates. Each bar indicates the standard error. We observed that the telomerase activity was also inhibited by genistein, but less effective than by apigenin in Fig. 3.

1997; Record, et al., 1997; Stoll, 1997). We examined the telomerase activity of SK-MEL 28 cells grown in DMEM-10% FBS containing 0, 45, 100, and 180 μM genistein, for 24, 48, 72 h, with the PCR ELISA method. As the control experiment, we also examined the telomerase of SK-MEL 28 cells grown in the serum-free media.

We observed that genistein also inhibited the telomerase activity in a dose-dependent manner (Fig. 4). The telomerase activity of SK-MEL 28 cells grown in the media genistein containing was inhibited, depending on the genistein concentration (from OD_{450} 3.7 to 2.5 for 48 h). However, the human melanoma telomerase was less inhibited by genistein than apigenin (Figs. 3 and 4). Even at the high genistein concentration (180 μM), the human melanoma telomerase was only slightly inhibited from OD_{450} 2.6 for 24 h to OD_{450} 2.3 for 72 h. SK-MEL 28 cell telomerase activity (OD_{450} 2.3) was inhibited at the two thirds of the maximum telomerase activity (OD_{450} 3.7) grown in normal growth condition (Fig. 4). At the same concentration of genistein (180 μM), we observed that the human melanoma telomerase was clearly inhibited by apigenin to OD_{450} 1.2. for 72 h (Fig. 3).

Induction of SK-MEL 28 cell apoptosis and the up-regulation of p53 by apigenin We next determined whether apigenin induced SK-MEL 28 cell apoptosis after treatment of SK-MEL 28 cell with apigenin. On the grounds that DNA fragmentation phenomenon is one of the typical cell apoptosis markers, we isolated genomic DNA and fractionated it on the agarose gel. We observed the apoptotic DNA ladder of SK-MEL 28 cells grown in DMEM 10% FBS containing apigenin (Fig. 5A). Thus, this data strongly suggest that apigenin also induced the programmed cell death of the human melanoma cell line. Because apigenin inhibited the cell growth, resulting in the apoptosis at the high concentration of apigenin for 24 h (Figs. 1 and 5A), we examined the expression of p53 in SK-MEL 28 cell with/without apigenin. In the Western blot, we observed p53 up-regulation by apigenin. p53 in the cell treated with apigenin, a tyrosine kinase inhibitor, increased 30 times of that in the control cell as measured by a densitometer (Fig. 5B). Thus, these data suggest that p53 expression, which is associated with SK-MEL 28 cell apoptosis, is also regulated by the tyrosine phosphorylation.

Discussion

We observed that apigenin inhibited SK-MEL 28 cell growth with morphological change (Figs. 1 and 2). Moreover, we explored that apigenin inhibited the telomerase (Fig. 3) and promoted apoptosis. Judging from our results in Fig. 5, the apoptotic pathway induced by apigenin seems to be correlated with p53 up-regulation.
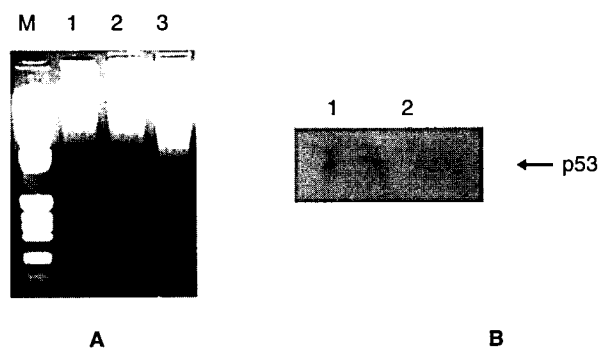
A   B

Fig. 5. The induction of SK-MEL28 cell apoptosis and the up-regulation of p53 by apigenin. A. SK-MEL 28 cells (1×10^6) were grown in 10% FBS–DMEM for 3 days and treated with 10% FBS DMEM containing 0, 45, and 100 μM apigenin for 24 h. The total genomic DNA was isolated by the procedure described in Materials and Methods. DNA ladder (1 kb) was used as the molecular marker. The genomic DNA fragmentation phenomenon of SK-MEL 28 cells, a typical apoptosis indicator, was observed at 100 μM apigenin. Lane M, DNA size marker; lane 1, without apigenin; lane 2, 45 μM apigenin; lane 3, 100 μM apigenin. B. The amount of p53 was measured by Western blot. SK-MEL 28 cells (1×10^6) were grown and treated with 10% FBS–DMEM containing 0 and 100 μM apigenin, under the same condition as described in Fig. 1. We observed 30-fold up-regulation of the p53 with the treatment of 100 μM apigenin (lane 1), over the control without apigenin (lane 2), as determined by a densitometric analysis.

However, it remains to be determined whether other proteins related with apoptosis, such as bcl-2 family proteins, are also regulated by apigenin.

In the serum-free media, we observed SK-MEL 28 cell growth without any further treatment (Fig. 1), and the up-regulation of telomerase activity in a time-dependent manner (Figs. 3 and 4). Thus, it is speculated that telomerase activation and cell growth are correlated with each other (Figs. 1, 3, and 4). We also noted that the secretion of interleukine-8 (IL-8), and not interleukine-6 (IL-6), from SK-MEL 28 cell is up-regulated in a time-dependent manner (unpublished data), together with the cell growth and telomerase activation (Figs. 1, 3, and 4). Thus, it seems that IL-8 secreted from the SK-MEL 28 cell plays a role for the autocrine function in the serum-free media (Krasagakis, et al., 1993; Shih and Herlyn, 1994).

Apigenin dramatically induced the inflammatory cytokine, IL-8, secretion from SK-MEL 28 cells in a time- and dose-dependent manner (unpublished data). The enhancement of IL-8 secretion by apigenin seems to be one of the reasons why the drug is not adequate as an anticancer drug in vivo, even though it has shown strong anticancer effect in vitro. Thus, it is possible that the enhancement of IL-8 secretion by apigenin induces an adverse inflammation response in the human body. It also remains to be determined whether the enhancement of IL-8 secretion by

apigenin stimulates the autocrine growth and metastatic potential of melanoma in vivo. However, we did not observe the up-regulation of IL-8 secretion by genistein, another tyrosine kinase inhibitor. In fact, genistein dramatically reduced IL-8 secretion from SK-MEL 28 cells (data not shown). Thus, the up-regulation of IL-8 secretion by apigenin appears to be its specific function which remains to be further characterized in detail.

For the first time, our results strongly demonstrate that apigenin inhibits SK-MEL 28 cell telomerase activity in time- and dose-dependent manners (Fig. 3). It still remains to be characterized whether the telomerase inhibition by apigenin is due to its specific function or the result of its cell growth inhibition. Since the telomerase activity of the cells grown in the serum-free media was higher than that of the cells grown in the normal growth condition (Figs. 1, 3, and 4), the telomerase inhibition seems to be not the result of the inhibition of cell growth. Recently, it has been reported that telomerase activity is regulated by protein phosphorylation in human breast cancer (Li et al., 1997). Moreover, we also observed the inhibition of the telomerase activity by both apigenin and genistein (Figs. 4 and 5). Thus, the inhibition of telomerase activity by these isoflavonoids appears to be due to its function as a tyrosine kinase inhibitor affecting the protein phosphorylation. We noted that the human melanoma telomerase was less inhibited by genistein than by apigenin (Figs. 4 and 5). Even at the high concentration (180 μM), the human melanoma telomerase was only slightly inhibited by genistein (Fig. 5), whereas the enzyme was clearly inhibited by apigenin (Fig. 4). Such differential effects of genistein and apigenin may be caused by the differences in the target protein kinase(s) which are specifically inhibited by each drug in the signal transduction pathway. Several studies have reported that apigenin inhibits the cdk2 kinase activity with the accumulation of the hypophosphorylated form of the retinoblastoma protein and the activation of the cdk inhibitor p21/WAF1 in a dose-dependent manner (Kuo and Yang, 1996; Lepley et al., 1996; Lepley and Pelling, 1997). However, the target protein kinase(s) of genistein is less clear. It has also been reported that genistein inhibits p56/p53lyn tyrosine kinase which phosphorylates Cdk1 on Tyr15 (Kaufmann, 1998). We are now investigating what kind of functional differences between genistein and apigenin cause the differential effect on the telomerase activity and IL-8 secretion from SK-MEL 28 cell line. Taken together, apigenin found in the plants can serve as a good anticancer drug to target telomerase, considering that telomerase is one of the interesting targets to control cell growth. However, before using as an anticancer agent, it should be considered that apigenin enhances the secretion of IL-8 from the cancer cells resulting in an inflammation response.

In summary, we have tested the effect of apigenin on SK-MEL 28 cell growth and the related protein expression

to explore how it affects on the human melanoma. Our results suggest that apigenin inhibits not only SK-MEL 28 cell growth but also its telomerase activity, with inducing apoptosis pathway related to p53. Therefore, apigenin abundantly contained in edible plants may play a role as an anticancer agent on the human melanoma, resulting in apoptosis.

References

Baek, J. H., Kang, C. M., Chung, H. Y., Park, M. H. and Kim, K. W. (1996) Increased expression of c-*jun* in the bile acid-induced apoptosis in mouse F9 teratocarcinoma stem cells. *J. Biochem. Mol. Biol.* (formerly *Korean Biochem. J.*) 29, 68–72.

Bestilny, L. J., Brown, C. B., Miura, Y., Robertson, L. D. and Riabowol, K. T. (1996) Selective inhibition of telomerase activity during terminal differentiation of immortal cell lines. *Cancer Res.* 56, 3796–3802.

Birt, D. F., Mitchell, D., Gold, B., Pour, P. and Pinch, H. C. (1997) Inhibition of ultraviolet light induced skin carcinogenesis in SKH-1 mice by apigenin, a plant flavonoid. *Anticancer Res.* 17, 85–91.

Brandi, M. L. (1997) Natural and synthetic isoflavones in the prevention and treatment of chronic diseases. *Calcif. Tissue Int.* 61 (Suppl. 1), S5–S8.

Brown, J. P., Nishiyama, K., Hellstrom, I. and Hellstrom, K. E. (1981) Structural characterization of human-melanoma-associated antigen p97 with monoclonal antibodies. *J. Immunol.* 127, 539–546.

Fotsis, T., Pepper, M. S., Aktas, E., Breit, S., Rasku, S., Adlercreutz, H., Wahala, K., Montesano, R. and Schweigerer, L. (1997) Flavonoids, dietary-derived inhibitors of cell proliferation and *in vitro* angiogenesis. *Cancer Res.* 57, 2916–2921.

Holt, S. E., Wright, W. E. and Shay, J. W. (1996) Regulation of telomerase activity in immortal cell lines. *Mol. Cell. Biol.* 16, 2932–2939.

Kang, S. S. and Folk, W. R. (1992) Lymphotropic papovavirus transforms hamster cells without altering the amount or stability of p53. *Virology* 191, 754–764.

Kaufmann, W. K. (1998) Human topoisomerase II function, tyrosine phosphorylation and cell cycle checkpoints. *Pro. Soc. Exp. Bio. Med.* 217, 327–334.

Kennedy, A. R. (1995) The evidence for soybean products as cancer preventive agents. *J. Nutr.* 125 (Suppl. 3), S733–S743.

Kim, N. W. and Wu, F. (1997) Advances in quantification and characterization of telomerase activity by the telomeric repeat amplification protocol (TRAP). *Nucleic Acids Res.* 25, 2595–2597.

Kim, T. U., Yang, S. H. and Kim, S. K. (1996) Cytotoxic and apoptotic effects of echinomycin on murine leukemia cells. *J. Biochem. Mol. Biol.* (formerly *Korean Biochem. J.*) 29, 489–493.

Klein, M., Weissenbock, G., Dufaud, A., Gaillard, C., Kreuz, K. and Martinoia, E. (1996) Different energization mechanisms drive the vacuolar uptake of a flavonoid glucoside and a herbicide glucoside. *J. Biol. Chem.* 271, 29666–29671.

Krasagakis, K., Garbe, C. and Orfanos, C. E. (1993) Cytokines in human melanoma cells: synthesis, autocrine stimulation and regulatory functions — an overview. *Melanoma Res.* 3, 425–433.

Kuo, M., L. and Yang, N. C. (1996) Reversion of v-H-ras-transformed NIH 3T3 cells by apigenin through inhibiting mitogen activated protein kinase and its downstream oncogenes. *Biochem. Biophys. Res. Commun.* 1212, 767–775.

Lee, M. H., Park, S. H., Song, H. S., Lee, K. H. and Park, J. S. (1997) A 100 kDa protein binding to bHLH family consensus recognition sequence of rat p53 promoter. *J. Biochem. Mol. Biol.* (formerly *Korean Biochem. J.*) 30, 205–211.

Lepley, D. M., Li, B., Birt, D. F. and Pelling, J. C. (1996) The chemopreventive flavonoid apigenin induces G2/M arrest in keratinocytes. *Carcinogenesis* 17, 2367–2375.

Lepley, D. M. and Pelling, J. C. (1997) Induction of p21/WAF1 and G1 cell-cycle arrest by the chemopreventive agent apigenin. *Mol. Carcinog.* 19, 74–82.

Levine, A. J. (1997) p53, the cellular gatekeeper for growth and division. *Cell* 88, 323–331.

Li, H., Zhao L. L., Funder J. W. and Liu, J. P. (1997) Protein phosphatase 2A inhibits nuclear telomerase activity in human breast cancer cells. *J. Biol. Chem.* 272, 16729–16732.

Plaumann, B., Fritsche, M., Rimpler, H., Brandner, G. and Hess, R. D. (1996) Flavoniods activate wild-type p53. *Oncogene* 13, 1605–1614.

Rauth S., Kichina J. and Green A. (1997) Inhibition of growth and induction of differentiation of metastatic melanoma cells *in vitro* by genistein: chemosensitivity is regulated by cellular p53. *Br. J. Cancer* 75, 1559–1566.

Record, I. R., Broadbent J. L., King, R. A., Dreosti, I. E., Head, R. J. and Tonkin, A. L. (1997) Genistein inhibits growth of B16 melanoma cells *in vivo* and *in vitro* and promotes differentiation *in vitro*. *Int. J. Cancer.* 72, 860–864.

Rhyu, M. S. (1995) Telomerase, telomerase, and immortality. *J. Natl. Cancer Inst.* 87, 884–894.

Shih, I. M. and Herlyn, M. (1994) Autocrine and paracrine roles for growth factors in melanoma. *In Vivo* 8, 113–123.

Stoll, B. A. (1997) Eating to beat breast cancer: potential role for soy supplements. *Ann. Oncol.* 8, 223–225.